The Effect of Paclobutrazol Treatment on Starch Content, Mycorrhizal Colonization, and Fine Root Density of White Oaks (*Quercus alba* L.)

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Abstract. Mature white oaks (*Quercus alba L.*) averaging 61 cm (24.4 in) dbh and in a moderate state of decline were treated with 0.8 g active ingredient (a.i.)/cm (2 g/in) paclobutrazol (PBZ) on 11 April 1995, and again with 1.2 g a.i./cm (3 g/in) on 8 October 1998, as a basal drench. PBZ produced no reduction in leaf size or twig growth of the white oaks at any time during the 7 years of the study. Rapidly growing plants may be more effectively growth-regulated by PBZ. Fine root density, starch content of the woody roots, and percentage of mycorrhizal root tips were unaffected by PBZ treatment. This is the first direct evidence that mycorrhizae are not reduced by the fungicidal properties of PBZ. The root zone of these trees was mulched but laterally restricted. Root density and mycorrhizae may have already been high under these conditions, limiting the ability of PBZ to improve them further.

Key Words. Carbohydrates; plant growth regulator; *Quercus alba*; tree growth regulator.

Gibberellin-inhibiting tree-growth regulators such as paclobutrazol (PBZ) have been shown to reduce shoot elongation, leaf expansion, and stem diameter growth of many tree species (Keever et al. 1990; Burch et al. 1996). More recently, PBZ has been shown to increase root growth of trees in certain landscape situations (Watson 1996, 2004; Watson and Himelick 2004). PBZ has promise as a tool for improving the health and vitality of urban trees.

Factors such as tree health and site quality may play a role in increased root development by PBZ. PBZ treatment did result in a significant increase in fine root density on chlorotic trees compared to untreated controls, but not on trees with normal foliage color (Watson and Himelick 2004). Fine root density of the healthy green trees may have been adequate, with little room for improvement. Poor soil conditions may limit response as well. Root density of chlorotic trees was improved by PBZ treatment in minimally disturbed golf course soils, but not along streets where soils are highly disturbed by the development process (Watson and Himelick 2004). Improvement of fine root density on white oak and pin oak also occurred in minimally disturbed soils (Watson 1996).

Too much crown growth regulation can reduce root development rather than enhance it. PBZ increased root extension growth of transplanted Green Column black maples (*Acer nigrum* 'Green Column') in the first year after treatment, prior to the onset of aboveground growth regulation, but not in the second year, when leaf and twig growth reduction was evident (Watson 2004). High twig and leaf regulation of elm seedlings leads to decreased root growth (Watson 2001).

Arborists have expressed concerns that mycorrhizae could be reduced by PBZ treatment because PBZ is closely related to triazole fungicides and exhibits fungicidal properties (Jacobs and Berg 2000). The current commercial formulations of PBZ available for urban forestry applications are applied as a basal soil injection or drench. Only a few roots at the base of the tree are in direct contact with the PBZ. PBZ is absorbed by roots and translocated in the xylem only, toward the branch tips, with little or no phloem mobility (Couture 1982). If PBZ is not transported to roots, it should have no fungicidal effect on mycorrhizal fungi, but this has never been confirmed.

Carbohydrate content of various plant tissues can be increased by PBZ (Wood 1984; Wieland and Wample 1985; Wang et al. 1986). Steffens et al. (1963) reported a 132% increase in total carbohydrates in fibrous roots of apple seedlings. Because mycorrhizal fungi receive carbohydrates from the plant as one of the major benefits of the symbiotic relationship, increased carbohydrates in the plant could result in increased mycorrhizae.

The objective of this study was to measure changes in fine root development, starch content, and mycorrhizal colonization of mature white oaks resulting from PBZ treatment in an urban landscape.

MATERIALS AND METHODS

Eighteen mature white oaks (*Quercus alba* L.) were selected on the grounds of the Morton Arboretum, Lisle, Illinois, U.S., near roads and buildings, similar to typical suburban settings. They were 42 to 76 cm (16.8 to 30.4 in) dbh [61 cm (24.4 in) average] located within 500 m (1,650 ft) of each other. All were in a moderate state of decline as judged by the amount of dieback, thinness, and/or chlorosis in the crown. Nearly all were growing in limited spaces and had well-established mulch over the root zone.

Nine of the trees were treated with PBZ. The PBZ concentrate was diluted with water per label instructions at 12.5:1 for a basal drench application with 0.8 g active ingredient (a.i.)/cm (2 g/in) on 11 April 1995, the same rate used in a previous study that increased root density of white oaks (Watson 1996). The product label calls for reapplication every 3 to 4 years. Due to a lack of response from the first treatment, all trees were retreated with a higher rate of 1.2 g a.i/cm (3 g/in) on 8 October 1998.

Sampling began in 1996. Each year in June, fine root development was measured using root density cores. One 20 cm (8 in) deep, 7 cm (2.8 in) diameter core was taken 1.5 m (5 ft) from the base of each tree. Cores were stored at 4°C (39°F) until processing. Soil was washed from the roots, and oak roots were separated from other roots and debris by hand. Length of fine roots [<2 mm (0.08 in) diameter] was measured and converted to fine root density with a WinRhizo system (Regent Instruments, Montréal, Québec).

June 2001 fine root samples were also used for assessment of mycorrhizal colonization. An additional set of core samples were collected in October 2001. Mycorrhizal colonization was assessed by staining fine roots with Ponceau S stain for 15 min at room temperature, destained with 10% acetic acid, and rinsed in deionized water. Root tips in ten randomly selected squares of a 625 square grid [2.5 mm (0.1 in) between lines] were examined under 20× magnification. Stained (red) root tips were recorded as ectomycorrhizal.

Woody roots 0.5 to 1.0 cm (0.2 to 0.4 in) diameter were collected from each tree for visual starch assessment in early June and early October, when starch reserves were expected to be at the low and high points in the normal annual cycle, respectively. At least two roots from each tree were used each season, more if they could be found without excessive disturbance. Cross sections were stained with I_2-KI solution and each sample was rated from 1 to 4 (devoid, low, medium, and high, respectively) (Wargo 1975).

Twig growth and leaf area were measured in August each year. An aerial lift was used to cut two samples per tree, from halfway up the crown on opposite sides. The distance from the budscale scars to terminal budtip was recorded in centimeters. Area of three fully expanded leaves was measured on a Delta-T (video) Area Meter (Delta-T Devices, Burwell, Cambridge, U.K.).

T-tests were used to compare twig growth, leaf area, and root densities each year ($P < 0.05$) using SigmaStat Version 3.0 for Windows.

RESULTS AND DISCUSSION

PBZ produced no reduction in leaf size or twig growth of the white oaks at any time during the 7 years of the study (Tables 1 and 2). High growth regulation was not desired because it could have resulted in a reduction, rather than stimulation, of root growth (Watson 2001, 2004). The 0.8 g a.i./cm (2 g/in) DBH rate used in the first treatment was chosen because it did result in increased fine root density in a previous study with white oaks (Watson 1996), but most of those trees had reduced, declining crowns. Trees in this study had fuller crowns, and the 0.8 g a.i./cm (2 g/in) DBH rate did not produce any measurable effects above or below ground.

Table 1. Twig growth (cm) of white oaks following PBZ treatments in April 1995 and October 1998.

Year	PBZ	Control
1996	7.2	6.7
1997	8.0	7.5
1998	6.1	7.8
1999	5.1	5.0
2000	10.4	11.0
2001	7.4	5.6
2002	7.6	8.5

There was no significant difference between treated and control trees in any year.

Choosing an appropriate rate is more difficult than it might seem. According to the product label, rate is determined by trunk diameter. Label rates for the 2SC formulation of PBZ can be traced back to the use for reducing aggressive regrowth of shoots after pruning near utility wires. Line-clearance pruning reduces crown size and over many years can result in smaller crown/trunk diameter ratios, similar to the declining trees in the earlier study (Watson 1996). The 0.8 g a.i./cm (2 g/in) dbh rate used for the first application, though current at the time, was lower than the rate recommended on subsequent revisions of the label. Given the higher crown/trunk diameter ratios of the trees in this study not repeatedly pruned for utility line clearance, or declining, the lack of growth regulation is understandable.

The lack of measurable growth regulation after the second treatment at a higher rate is more puzzling. Greener leaves (observation only), especially on chlorotic trees, were evidence that PBZ was affecting the crowns. Most studies on

Table 2. Leaf area (cm^2) of white oaks following PBZ treatments in April 1995 and October 1998.

Year	PBZ	Control
1997	58.2	52.9
1998	45.2	51.1
1999	51.6	47.0
2000	41.6	43.1
2001	37.9	41.0
2002	45.8	45.1

There was no significant difference between treated and control trees in any year.

growth regulation with PBZ are on moderate-sized, heavily pruned trees with rapid sprouting (utility and orchard), or small vigorous plants. Annual twig growth on these large mature trees was typically only 5 to 8 cm (2 to 3.2 in) per year. Growth regulation by PBZ may be less effective on slowly growing plants. More research is needed on the use of PBZ on large trees.

Except for one year, 1998, fine root density was not increased in this study (Table 3) as it had been in others, including one study using similar size and species trees treated with a similar rate. Greening was present, indicating the growth regulator was present in the leaves, especially after the second application, but there was no overregulation of top growth that could have reduced root growth. The 1 year increase is probably not horticulturally significant, and the reason for it is unclear.

Starch content of the woody roots was not affected by PBZ in either season. Visual assessment of starch is not as precise as chemical methods used to detect carbohydrate changes in previous studies but can detect biologically important differences that could affect tree health and vitality (Wargo 1975). In June, starch levels were low but not depleted. It is possible that reserves started to rebuild from the lowest levels by that time, as they did for sugar maple in the northeast (Wargo 1979). Stored starch levels did not increase by October, but the reason for this is not clear. There was no unusual weather pattern or defoliation that may have reduced photosynthesis, nor was there any unusual crown growth that might have depleted reserves.

Mycorrhizal colonization of roots also was not affected by PBZ (Table 4). Without an increase in carbohydrates available in the roots, and/or changes in the soil environment, a lack of change in mycorrhizal colonization of root tips is understandable. This is the first direct evidence that mycorrhizae are not reduced by PBZ and is in agreement with previous reports that basally applied PBZ is not transported to the root tips, where the fungicidal properties could affect the mycorrhizal fungi (Couture 1982). The percentage of mycorrhizal root tips appears to be lower in June than October, but this may simply have been the result of a different technician performing the visual color assessment.

Table 3. Root density (cm/cc soil) of white oaks following PBZ treatments in April 1995 and October 1998.

Year	PBZ	Control
1996	1.23	1.46
1997	1.40	1.76
1998	2.31*	1.26
1999	3.71	3.55
2000	3.32	2.35
2001	1.64	1.68
2002	1.92	1.75

*Significantly different from control ($P \leq 0.05$)

Table 4. Stored starch and mycorrhizal colonization of roots of white oaks in the third season after treatment with PBZ.

	PBZ	Control
Starch		
June	2.0	2.5
October	2.4	2.6
Ectomycorrhizae (% of root tips)		
June	38%	33%
October	76%	71%

There was no significant difference between treated and control trees in any year.

The root environment for the majority of these trees was of high quality, with a well-established mulch layer, but limited in quantity, with lateral spread restricted by pavements and structures. Root density and mycorrhizae may have already been maximized under these conditions before the PBZ treatments. Mulching with wood chips has been shown to increase root density and mycorrhizal colonization of the roots of white oak trees (Himelick and Watson 1990). It is possible that in a very favorable root environment, fine root density and mycorrhizal colonization were already high and PBZ was not able to improve them further. Though in some situations PBZ can improve the root growth and crown appearance of declining trees (Watson 1996), this study indicates that the conditions under which PBZ can be effective may be quite specific. More research is needed to understand under what conditions PBZ treatments can be used by arborists to improve fine root development and help to stabilize declining trees.

Acknowledgments. This study was funded in part by the Care of Trees, Wheeling, Illinois; Rainbow Treecare Scientific Advancements, St. Louis Park, Minnesota; and Dow AgroSciences, Indianapolis, Indiana.

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Résumé. Des chênes blancs matures (*Quercus alba* L.) de 61 cm de DHP en moyenne et dans un état de dépérissement modéré ont été traités avec 0,8 g/cm d'ingrédient actif de paclobutrazol le 11 avril 1995 et à nouveau avec 1,2 g/cm le 8 octobre 1998, et ce par application au niveau du sol. Le paclobutrazol n'a produit aucune réduction dans la dimension des feuilles ou la croissance des pousses chez le chêne blanc, et ce à n'importe quel moment de l'étude menée sur une période de sept ans. Les végétaux à croissance rapide peuvent être plus facilement contrôlés dans leur croissance par le paclobutrazol. La densité en fines radicelles, le contenu en amidon dans les racines lignifiées et le pourcentage de racines mycorhizées n'étaient pas affectés par le traitement avec le paclobutrazol. Ceci constitue la première preuve concrète que la présence de mycorhizes n'est pas diminuée par les propriétés fongiques du paclobutrazol. La zone d'enracinement de ces arbres était recouverte de paillis, mais avec une limitation latéralement. La densité en racines et en mycorhizes pourrait avoir été déjà élevée en regard de ces conditions, ce qui aurait alors limité la capacité du paclobutrazol à l'améliorer encore plus.

Zusammenfassung. Am 11 April 1995 wurden Weißeichen mit einem durchschnittlichen BHD von 61 cm und in einem leicht kranken Zustand mit 0,8g Paclobutrazol (PBZ) als Flüssiggabe an der Stammbasis behandelt. Am 8. Oktober 1998 wurde die Behandlung mit 1,2g Paclobutrazol wiederholt. PBZ produzierte während der 7 Jahre der Studie bei keiner Eiche eine Reduktion der Blattgröße oder Zweiglänge. Rasch wachsende Pflanzen mögen durch PBZ effektiver im Wachstum reguliert werden können. Die Dichte der Feinwurzeln, der Stärkegehalt der holzigen Wurzeln und der Prozentsatz der Mycorrhiza-Wurzelspitzen wurden nicht durch PBZ beeinflusst. Dies ist der erste direkte Beweis, dass Mycorrhiza nicht durch die fungiziden Anteile von PBZ beeinträchtigt werden. Die Wurzelzone dieser Bäume war gemulcht, aber seitlich begrenzt. Die Wurzeldichte und Mycorrhiza hääte unter diesen Bedingungen schon hoch sein können, was die Möglichkeit des PBZ, sie zu unterstützen, begrenzt hätte.

Resumen. Encinos blancos maduros *(Quercus alba L)* promediando 61 cm. de DAP en un estado moderado de declinación fueron tratados con 0.8 g de ingrediente activo (a.i.)/cm. (2g/in) de paclobutrazol (PBZ) en Abril 11, 1995, y de nuevo con 1.2 g a.i/cm. (3 g/in) en Octubre 8, 1998 a través de una zanja basal. El PBZ no produjo reducción en el tamaño de la hoja o crecimiento de brotes de los encinos en cualquier momento durante los siete años del estudio. El crecimiento rápido de las plantas puede ser más efectivamente regulado por PVZ. La densidad de raíces finas, contenido de almidones de las raíces leñosas y el por ciento de raíces mocorrízicas no fueron afectados por el tratamiento de PBZ. Esta es la primera evidencia directa de que las mocorrizas no son reducidas por las propiedades fungicidas de PBZ. La zona radicular de estos árboles fue mulcheada pero lateralmente restringida. La densidad de las raíces y micorrizas puede llegar a ser alta bajo estas condiciones, limitando la habilidad del PBZ para mejorarlas.